# SOLID PHASE MICROEXRACTION – SPME TROUBLESHOOTING GUIDE

## I- SPME product related

SPME fiber breakage is a potential problem when you apply excessive stress to the fiber during sampling or analysis.

SPME accessories such as the inlet guide, sampling stand, heat/stir plate, magnetic stirring bars, and thermometer will improve the reproducibility and ease of the sampling and desorption steps.

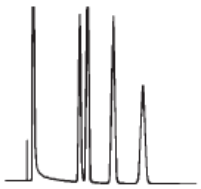
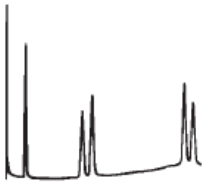
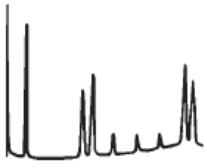
Do not expose PDMS coated fibers to non-polar solvents and do not expose Carbowax coated fibers to polar solvents. The fiber coating will swell and cause damage may include breakage, grooving, or stripping of the fiber coating. To avoid this problem, dilute the sample with water before extraction to reduce the organic solvent percentage to less 3%.

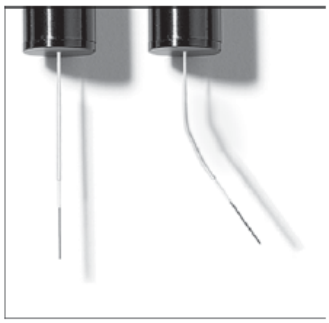
| Film thickness | Description | Hub description | Recommended use |
|---|---|---|---|
| **Polydimethylsiloxane (PDMS)** | | | |
| | – considered non-polar for non-polar analytes | | |
| 100µm | non-bonded | red/plain | GC / HPLC |
| 30µm | non-bonded | yellow/plain | GC / HPLC |
| 7µm | bonded | green/plain | GC / HPLC |
| **Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)** | | | |
| | – ideal for many polar analytes, especially amines | | |
| 65µm | partially crosslinked | blue/plain | GC |
| 60µm | partially crosslinked | brown/notched | HPLC |
| 65µm StableFlex* | highly crosslinked | pink/plain | GC |
| **Polyacrylate** | | | |
| | – highly polar coating for general use, ideal for phenols | | |
| 85µm | partially crosslinked | white/plain | GC / HPLC |
| **Carboxen/Polydimethylsiloxane (CAR/PDMS)** | | | |
| | – ideal for gaseous/volatile analytes, high retention for trace analysis | | |
| 75µm | partially crosslinked | black/plain | GC |
| 85µm StableFlex* | highly crosslinked | light blue/plain | GC |
| **Carbowax/Divinylbenzene (CW/DVB)** | | | |
| | – for polar analytes, especially for alcohols, low temperature limit | | |
| 65µm | partially crosslinked | orange/plain | GC |
| 70µm StableFlex* | highly crosslinked | yellow-green/plain | GC |
| **Carbowax/Templated resin (CW/TPR)** | | | |
| | – developed for HPLC applications, *e.g.* surfactants | | |
| 50µm | partially crosslinked | purple/notched | HPLC |
| **Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS)** | | | |
| | – ideal for broad range of analyte polarities, good for C3-C20 range | | |
| 50/30µm StableFlex* | highly crosslinked | gray/plain | GC |
| 50/30µm StableFlex* | highly crosslinked | gray/notched | GC |

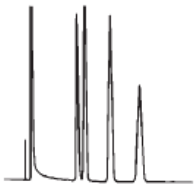
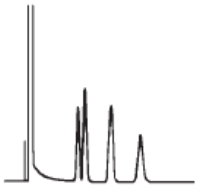
In the previous table, StableFlex* = StableFex (Supelco®) fiber is more flexible than original fibers. It is designed to minimize fiber breakage.

## II- Troubleshooting table

See in the table, A = Analysis related
D = Desorption related
P = Product related
S = Sampling related

| Problem / Symptom | Possible Cause | Remedy |
|---|---|---|
| 1. No peaks seen in GC analysis<br>  <br>Normal<br>  <br>Problem | 1. Instrument problems (A)<br>2. The splitter vent was left open (D)<br>3. The analyte concentration is too low to be detected (S)<br>4. Solvents present in sample competing with SPME extraction (S)<br>5. Headspace volume too large to establish equilibrium with fiber (S)<br>6. Coating on fiber deteriorated (P)<br>7. Incorrect SPME fiber used for extraction (P)<br>8. There is a leaking injection port (septum or connection) (D)<br>9. There is a leaking sample vial (S)<br>10. Loss during transport from the field (S) | 1. Inject standard mixture to verify detector response, see GC troubleshooting guide for help<br>2. Run splitless injection for 2 min.<br>3. Start with known concentration (1ppm) of analyte in de-ionized water mixture. Optimize extraction by adjusting extraction time, temperature, and chemical condition of pH and salt<br>4. Minimize solvents in the sample to <3% by dilution in water<br>5. Reduce headspace to 50% or less, agitate sample vigorously, or increase the sampling temperature.<br>6. Replace fiber. Fibers are reusable and will last for 50 injections on average.<br>7. This is beyond the scope of this guide. Please contact technical service (800-359-3041 / 814-359-3041) for assistance if you are experiencing problems selecting the appropriate fiber for your application<br>8. Replace septum and tighten nut properly<br>9. Replace vial septum and seal cap properly<br>10. Move the depth adjusting lever of the portable field sampler to the top-most locking slot, so the end of the septum-piercing needle is totally withdrawn into the sealing septum of the sampler. If the fiber will be stored for more than one day we recommend that it be stored at subambient temperature. This reduces the chance of breakdown and oss of sample that could occur at higher temperatures. |
| 2. Extraneous peaks in analysis<br>  <br>Normal<br>  <br>Problem | 1. Septa used in sampling vial or injection port is outgassing organic contaminants (S/A)<br>2.Fiber is not preconditioned prior to sampling (P)<br>3. Inlet liner is contaminated or contains septa particles (D)<br>4. GC column is collecting analytes on the front of the column because it is not heated high enough in sample analysis (A)<br>5. Interfering peaks coelute with analytes of interest (A)<br>6. Carryover from previous analysis of the fiber (P)<br>7. Cross-contamination from laboratory air (S)<br>8. Cross-contamination during transport from the field (S) | 1. Prebake the vial septa for 2 hours at 68°C prior to use. Use low-bleed LB-2 septa to minimize injection port septum bleed.<br>2. Precondition fiber at the recommended conditioning temperature in the fiber instruction sheet. Once fiber is preconditioned, only 1-2 minutes is required to clean the fiber prior to sampling.<br>3. Replace the inlet liner. Use pre-drilled septum or a septumless injector system (e.g. Merlin Microseal)<br>4. Complete GC analysis temperature program before injecting another SPME extract and keep column at 150°C when not in use.<br>5. Change GC column or temperature program rate<br>6. Bake out fiber at the recommended conditions for several additional minutes<br>7. Do not expose the fiber to the laboratory environment at any time during the sampling or injection steps. Analyze control blanks using the same handling process as the sample to determine if technique or laboratory cross-contamination is present.<br>8. Move the depth adjusting lever of the portable field sampler to the top-most locking slot, so the end of the septum-piercing needle is totally withdrawn into the sealing septum of the sampler. If the fiber will be stored for more than one day we recommend that it be stored at subambient temperature. This reduces the chance of sample cross-contamination. |

| Problem / Symptom | Possible Cause | Remedy |
|---|---|---|
| 3. Fiber will not retract or sticks in holder needle | 1. The end of the needle is plugged with a piece of septum (D) | 1. Injection port septum nut is overtightened. Loosen the nut slightly to allow for improved injection. Use pre-drilled injection port septa or a septumless injector system (e.g. Merlin Microseal) |
| | 2. The fiber was exposed to solvents that caused swelling of coating (S) | 2. Do not expose PDMS coated fibers to non-polar solvents such as pentane, methylene chloride, or diethyl ether. Do not expose Carbowax fibers to polar solvents. |
| | 3. The top screw in the holder assembly is too tight (P) | 3. Loosen the top screw on the holder assembly slightly to allow for free movement of the plunger. |
| 4. Needle bends during injection into sample vial or GC injection port  | 1. Improper manual sampling technique (S) | 1. To prevent the needle from bending when doing manual SPME sampling, follow this procedure:<br>• Adjust the SPME needle to the 0.2 depth gauge setting on the plunger (first tick mark). This will expose about 3mm of the needle through the end of the black holder.<br>• Hold the SPME assembly on top of the sampling vial with the bottom of the black holder flush with the top of the vial cap.<br>• Hold the sampling vial and black SPME holder base securely with one hand<br>• Twist the stainless steel plunger clockwise with the other hand<br>• Keep turning the plunger until the desired depth setting is achieved (you will usually hear a pop when the needle pierces the septum).<br>• Expose the fiber and perform sampling as usual |
| | 2. Improper manual desorption (injection) technique (D) | 2. To prevent the needle from bending when doing manual SPME injections, follow this procedure:<br>• Adjust the SPME needle to the 0.2 depth gauge setting on the plunger (first tick mark). This will expose about 3mm of the needle through the end of the black holder.<br>• Position the SPME assembly on top of the GC injection port or in the SPME inlet guide with the bottom of the black holder flush with the top of the injector or guide.<br>• Hold the black SPME holder base securely with one hand<br>• Twist the stainless steel plunger clockwise with the other hand<br>• Keep turning the plunger until the desired depth setting is achieved (you will usually hear a pop when the needle pierces the septum).<br>• Expose the fiber and perform sample desorption as usual |
| | 3. Vial or injection port septum is too tight (S/D) | 3. Loosen slightly the vial closure or injection port nut |
| | 4. Septa in sample vial/injection port is too thick or coated with thick Teflon® coating (S/D) | 4. Use LB-2 septa for injection port or silicone septa with <10mil Teflon on sampling vials. Shorten the amount of exposed needle on SPME holder to 0.5 inch or (~1cm) before puncturing the vial septa. Adjust the holder needle setting to the desired depth for sampling. Do not use butyl rubber style septa. |
| | 5. GC inlet liner is too narrow or packed with adsorbent material (D) | 5. Use larger splitless inlet liners (0.75mm ID or larger) without glass wool or adsorbents. |
| Needle bends with automated injection systems | 6. Needle is out of alignment with injection port or sample vial (S/D) | 6. Reference autoinjector manual on alignment |

| Problem / Symptom | Possible Cause | Remedy |
|---|---|---|
| 5. Fiber breaks | 1. The fiber was not retracted into the protective needle after removal from sample vial or injection port (S/D)<br>2. The end of the needle is plugged with a piece of septum (D) | 1. Retract fiber into protective needle during insertion into vial/injection port and removal<br>2. Injection port septum nut is overtightened. Loosen the nut slightly to allow for improved injection. Use pre-drilled injection port septa or a septumless injector system (e.g. Merlin Microseal) |
| 6. Reproducibility is poor<br>  <br>Normal<br>  <br>Problem | 1. Time and temperature variations during sampling (S)<br>2. Not consistently positioning the fiber at the same depth during sampling (S)<br>3. pH or salt conditions varying during sampling (S)<br>4. Equilibrium is not reached during extraction (S)<br>5. Varying organic content in the samples (S)<br>6. Varying headspace in sample vials during headspace extraction (S)<br>7. Solid samples not releasing analytes for extraction (S)<br>8. Competing analyte displaces compound of interest/or interferes (S)<br>9. Not reproducing desorption conditions (D)<br>10. Not using agitation during sampling or apply it inconsistently (S)<br>11. Sample volumes are inconsistent (S) | 1. Time of extraction and temperature are the two most critical conditions to control. Use timing device and calibrated thermometer to ensure reproducible results. Remember that room temperature fluctuations will influence the ambient sample temperature.<br>2. Position fiber just below sample surface for immersion sampling and at a consistent position above the sample during headspace sampling.<br>3. Apply uniformly across all extractions any pH or salt adjustments made to the samples.<br>4. Determine minimum time for equilibrium using a standard mixture and controlled extraction conditions. Note that full equilibrium is not required to be reached for all applications to achieve reproducible results.<br>5. Dilute samples to minimize solvent interference or use headspace sampling to minimize solvent effect. Use internal standards, surrogates, or the standard addition technique to compensate for variations in sample matrix.<br>6. Minimize headspace volume to 50% or less and agitate the sample. Maintain the same headspace volume and agitation conditions across all extractions.<br>7. Grind solid into small particles, add to water, and apply heat and agitation.<br>8. Reduce the extraction time to minimize displacement/or interference<br>9. Verify that the fiber position (depth), desportion time, temperature, and splitless conditions are consistent. Use an automated SPME system to improve reproducibility.<br>10. Use a stir bar or soncication system to agitate the sample during sampling. Maintain consistent agitation conditions for all standards and samples.<br>11. Maintain consistent volumes for all standards and samples. |

| Problem / Symptom | Possible Cause | Remedy |
|---|---|---|
| 7. Fiber discolored | 1. Fiber is oxidized during fiber conditioning or sample injection into GC (S/D)<br>2. Heating during injection (D/P) | 1. Minimize oxygen in carrier gas, condition fibers in oxygen free gas flow. Reduce the injection port temperature to the recommended maximum setting. Carbowax/DVB coatings are especially sensitive to temperature (<260°C is recommended).<br>2. Does not usually affect the performance of the fiber. Always minimize the oxygen content in the GC carrier gas to avoid oxidizing the fiber coating. The polyacrylate coated fiber will discolor above 280°C. Carbowax/DVB may slightly darken during use, however, if the fiber turns brown, lower the injection port temperature (265°C is the recommended maximum) and check the system for leaks. |
| 8. Number of injections from the fiber is less than previously obtained | 1. Fiber is oxidized during fiber conditioning or sample injection into GC (S/D)<br>2. Coating on fiber deteriorated (P)<br>3. Fiber was exposed to solvents that cause swelling of coating (S) | 1. Minimize oxygen in carrier gas, condition fibers in oxygen free gas flow. Reduce injection port temperature to the recommended fiber maximum.<br>2. Replace fiber. Fibers are reusable and will last for 50 injections on average.<br>3. Do not expose PDMS coated fibers to nonpolar solvents such as pentane, methylene chloride, or diethyl ether. Do not expose Carbowax fibers to polar solvents. |